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Bluestem of the Black Raspberry

By W. H. LAWRENCE

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BLUESTEM OF THE BLACK RASPBERRY.

By W. H. LAWRENCE.

(Formerly Plant Pathologist and Superintendent, Western Washington Station.)

The adaptability of the Puget Sound country to the growing of bush fruits has long since been demonstrated. With the growth of the industry many new, as well as all the standard, sorts of raspberries and blackberries were given trials by various growers of bush fruits. Among the raspberries introduced, a few of the black caps gave great promise. The excellent quality and flavor of the berry won for it great favor on the local markets and at much more remunerative prices than the red raspberry or blackberry. To supply the demand, increased plantings were made.

During 1904 to 1907 considerable complaint was received concerning the behavior of individual hills of black raspberry bushes. For some unexplained reason some of those failed to mature their load of fruit properly or died before harvest time. Conditions became more serious until in 1907, the total shipments made by the Puyallup and Sumner Fruit Growers' Association and the Union Berry Shippers did not exceed 150 crates. Although the demand was excellent, and the price offered more than double that for other raspberries, sufficient quantities could not be had to supply the market. Since the growers fully appreciated the behavior of this sort of raspberry, it was quite natural that information relative to the successful commercial production of the crop was largely sought. Since the behavior of these plants was an unsolved problem, the writer made a field survey covering the areas where greatest loss had occurred. The observations showed plainly that the time and condition of the soil had little influence on the severity of the trouble, nor was there any variety of the black cap immune even when grown under the best conditions of soil and cultivation.

Preliminary pathological investigations were begun. Inoculation experiments were made to prove the pathogenisity of the fungus which could be had in pure cultures secured by transferring small portions of the stems to culture media. A very limited amount of work was also done in attempting to prevent further loss in one field by saturating the soil and thoroughly wetting the crowns of plants with a fungicide.

A careful study of the fungus and its behavior in culture media was made by the writer during the year 1907-08 while working in the botanical laboratories of Cornell University. Additional field observations upon the disease and steps toward the solution of the problem were undertaken in 1909 and 1910. The writer having severed his connection with the Experiment Station under whose direction this work was being conducted, this report is made as a working basis for future investigation of this disease.

SOURCES AND VARIETIES OF BLACK RASPBERRIES VERSUS THE DISTRIBUTION OF INFESTED VARIETIES.

Since the kind and condition of soil had little influence on the severity of the disease, as all diseased canes failed to mature good fruit, and all varieties were found to be more or less infested, an investigation of the sources and varieties introduced was made. At this time nearly every fruit grower in the valley had at least a few bushes of some sort of black cap, so that observations covering these points were quickly made. It was found that practically every known variety of black cap had been introduced from some part of the United States. Evidences of the presence of the fungus in all fields except one indicated that the disease could not have been introduced in all instances at least. Since the disease was prevalent in all types of soils, it was concluded that the fungus must live in the soil. Its distribution is general throughout the Puget Sound country, since not only the introduced varieties but the native wild black cap is subject to the same disease both when transplanted in the home gardens and as it grows wild in the clearings and burned-over areas. Furthermore, this fungus has twice been taken from the roots of the Antwerp red raspberry and once from the roots of a Snyder blackberry bush, which had died from some unknown cause.

CHARACTERS OF THE DISEASE SHOWN BY THE STEMS, LEAVES, FRUIT AND ROOTS.

General Characteristics of the Disease.

Usually the canes, especially those bearing the heavy crops of fruit, give the first evidences of the disease. The fruit begins to dry up and become woody about the time it is partially mature in size. This change in the fruit is usually accompanied by a more or less conspicuous discoloring and wilting of the leaves, and a darkening in the shade of color of the canes themselves. In rare cases the color of the cane changed from the

normal to a blue-black, which is usually most conspicuous toward the base and is more pronounced in those canes which have made the greatest wood growth. Very rarely the canes become striped, usually however, gradually changing to a deeper shade of green or blue. The severity apparently depends upon the length of time the cane has been infested, the abundance of the fungus and the succulency of the canes themselves, since every

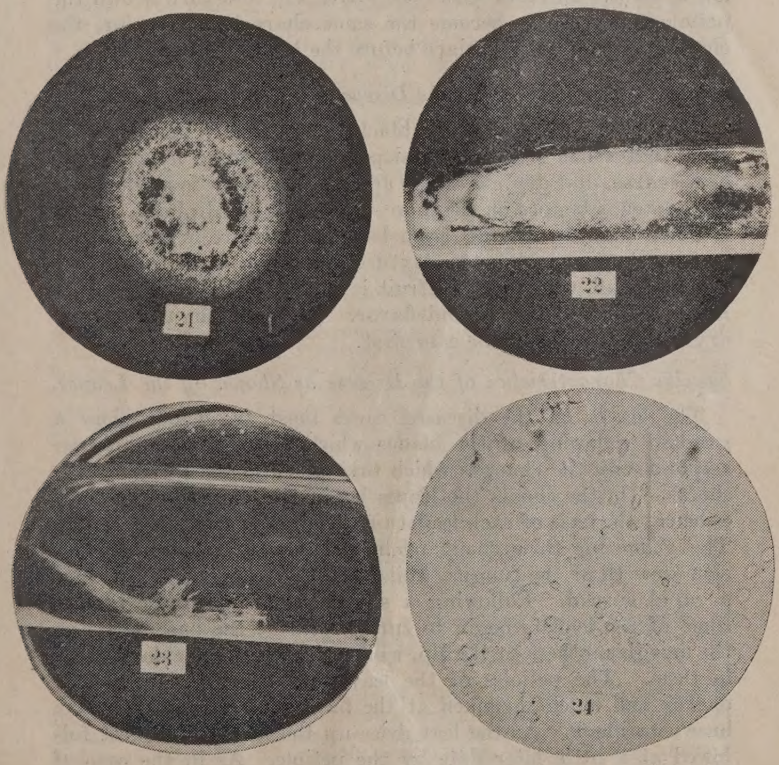


FIG. 21.—From a photograph of a colony of A. caulophagus showing the nature of the fertile hyphae. This colony was grown from a single spore transplanted after it had produced a mycelium visible to the naked eye. Attention is called to the dense growth made at the point of inoculation and the weaker but more evenly flocose development over the remainder of the colony. The zonated growth usually made by this fungus is also evident in this specimen.

FIG. 22.—Growth of the fungus in the test tube. Source of inoculation was a diseased cane.

FIG. 23.—Fascicles of A. caulophagus produced on potato agar.

FIG. 24.—Micro-photograph of conidia showing variations in size and shape.

degree of injury may be observed, ranging from a total loss of the crop of fruit to well formed and pulpy but tasteless fruit. The action of the disease in the canes is slow as compared with that in the shoots, where, in most cases, it is extremely rapid. In the shoots the blue-black color may appear and spread over the entire canes in the course of a few days, during which time, the leaves wilt and turn brown, or gradually become light yellow in color. In cases where the leaves wilt and turn brown the petioles many times become the same characteristic color, the change in color taking place before the blades fall off.

Specific Characteristics of the Disease as Shown by the Fruit.

The diseased fruit of the black cap is easily distinguished from that which is healthy and properly matured by its tasteless, coarse, and dry character and the partially formed woody and tasteless berries on the more severely injured plants. Poor fruit on plants suffering from lack of moisture or plant food usually show a much better texture and flavor, even though the fruit is small, while healthy fruit is characterized by large berries of excellent texture and flavor. Diseased berries begin to dry up on the tips of the cane first.

Specific Characteristics of the Disease as Shown by the Leaves.

The leaves on the diseased canes most frequently show a gradual yellowing of the blades which may or may not correspond with the changes which take place in the leaves on the shoots. On the shoots the leaves begin to turn yellow in color either at the base of each leaflet or throughout the entire leaflet. The yellowing throughout probably indicates a gradual but slow growth of the fungus, while the other conditions represent a rapid spread. Following a slight yellowing of the blades, some of the leaflets begin to turn brown at the base, some on the margins, others at the tip, while still others have dark areas in them. The petioles of the leaves in the meantime become darker and much shrunken at the base, later turning to dark brown to black. As the leaf dries up the leaflets fall first followed at a little later date by the petiole. As in the case of the drying up of the fruit, the leaves first show signs of wilting and most rapid drying from the tip downward.

Characteristics of the Disease as Shown by the Shoots.

Since the characteristics of the disease as shown by the external condition of the infested stems vary so greatly, a description of a number of shoots of the Cumberland black raspberry

is here presented, in order that the nature of the injury may be more easily recognized. The observations were all made on the same day, thus giving the comparative condition of the plants on August 16th.

In one shoot, 5.5 feet tall, there appeared a blue-black stripe one-fourth of an inch wide and forty inches long, beginning at a point six inches above the ground line, with both ends of the stripe terminating in fringed ends of gradually fading streaks of blue-black, receding into reddish brown, the longest and most conspicuous being located at the centers. The ends of stripes generally present this condition of discoloration, but in most cases are less conspicuous and definite than in this specimen. The rich green of the healthy portion of the stem in contrast with the blue-black stripe covered with the bloom which is so abundant and so characteristic of the Cumberland, made the diseased portion extremely conspicuous. This hill contained four shoots. The bearing canes, four in number, produced a large yield of excellent fruit.

In a hill of four shoots, one of them beginning at the crown below the surface of the ground, the first six inches was entirely discolored on one side and was well covered with scattering, small deep blue blotches on the remainder. For a distance of 22 inches upward the bark was dark blue to black throughout, the color being deeper and more pronounced toward the base. From this discolored portion of the cane the leaves had fallen, the leaf scars of which were very conspicuous as the scars and immediate surrounding tissues were of a normal color. Beginning at the point where the cane was entirely discolored over its entire circumference, the color extended an additional 25 inches, gradually narrowing above, and ultimately terminating as a narrow stripe with a distinct V-shaped apex. The boundary between the normal and discolored portions, while rather irregular, was quite distinct. The leaves had also fallen from this portion of the shoot. Some of the leaf scars were green while others were blue. The remaining portion of the shoot was apparently normal. One of the four shoots was entirely dead. It was well coated with the bloom, the color varying from a deep blue to a black-blue. After the bloom was rubbed off, the color varied from dark reddish brown to black. Beginning at the base, the stem presented a longitudinally striated appearance, which gradually became less distinct toward the apex. This appearance was due to the shrinking of the cortex of the bark. The remaining two shoots as far as the eye could detect were perfectly healthy.

In a third case, the shoot was a blue-black for a distance of 42 inches on one side only, the stripe being of equal width throughout. The first visible discoloration at the base occurred six inches from the ground line.

In a rank growing shoot a lateral branch 36 inches long located 19 inches above the last visible discoloration of the lower portion, was discolored for more than half its length. An examination of the bark and wood showed a few reddish brown lines, extending from the discolored area below into the base of the branch above. A microscopic examination of this wood revealed numerous hyphae which in some of the tracheae became so abundant and closely wedged together to form a perfect plug or cork. (See Figs. 43-44.)

Another large and branched shoot was covered with yellow leaves, the petioles of which were mostly discolored. Two branches and the main shoot were blue-black throughout for a distance of 52 inches. A few leaves above the point of discoloration were variously wilted while

those above were in normal condition with the exception of a slight change of color.

A sixth cane observed was discolored for a distance of 15 inches from a point 4 inches above the base.

A very unusual condition was observed in one shoot which had a blue girdle one inch in width near the base, was normal in color externally 4.5 inches, above which there was a colored stripe one-fifth the circumference of the cane and 38 inches long, 30 inches of which were unusually deep colored, gradually fading and entirely disappearing in a distance of eight inches, the color again appearing throughout the entire length of a 15-inch branch located 8 inches higher up the stem.

In another hill one of the shoots was discolored from near the base to the apex on one side, on the lower 20 inches of which the

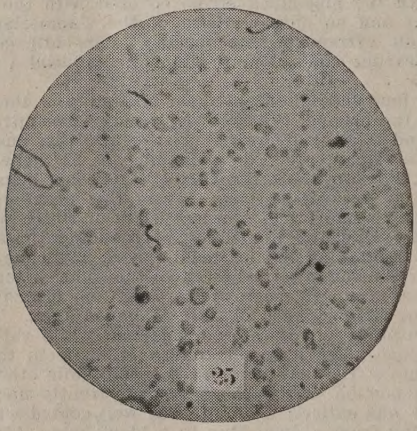


FIG. 25.—Micro-photograph of asci-like cells developed in the cultures showing yeast-like growth and emitting a yeast-like odor. Special attention is called to the spore-like bodies appearing in pairs bounded by an indistinct line which represents a cell wall.

leaves were entirely discolored in the petioles or dead and discolored throughout.

A ninth shoot representing a usual condition had its lower three feet entirely black throughout. Still another shoot showed the same condition throughout the lower 26 inches, above which occurred a stripe on one side for a distance of 21 inches. The stripe was very distinct and conspicuous since it did not cover more than one-fourth the circumference of the stem.

The next shoot examined was discolored on one side only for a distance of 28 inches.

A very unusual condition was observed in which a blue stripe one-eighth of an inch wide, began at the crown and extended up one side a distance of 43 inches, becoming invisible at several places. There were also numerous blue specks over the lower two feet of the cane with a few scattering ones up as high as the stripe extended, the latter ending at the base of a lateral ten inches in length, which was discolored throughout. In this case, as is generally true where the canes are discolored only on one side, the margin is broken with numerous adjacent red specks which are more or less depressed.

Beginning at the base a stripe 25 inches in length extending one-third around the stem gradually became invisible to the eye but again appeared in each of three branches 12, 16, and 24 inches from the last point of discoloration, each of which was in turn discolored for a greater portion of their length.

Another shoot was conspicuous by the presence of a narrow stripe which began at the base and extended up the side to a large lateral two feet above. The stripe extended into this branch a distance of two feet and also a similar distance up the side of the shoot. Half the surface above the branch was discolored while below the stripe was somewhat narrower. On the side opposite the stripe on the lower three feet of the stem occurred numerous small spots which were particularly conspicuous where the epidermis had split open at earlier dates.

Contrary to the usual occurrence, the discoloration which took place in this specimen first appeared ten inches above the crown, at which

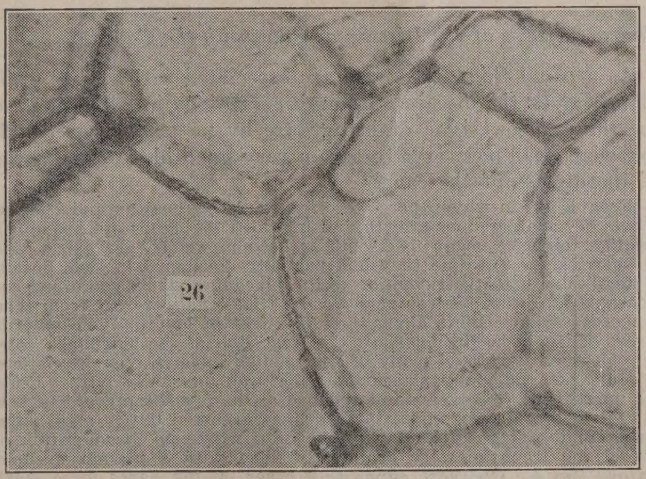


FIG. 26.—Micro-photograph and cross section of the pith of blackberry stem showing a few hyphae crossing the cells.

point a narrow stripe extended upward 36 inches. There were also a number of widely scattered blue-black spots on the lower portion of this cane. This specimen also showed some very curious exceptions in discoloration. The change in color occurred over the entire stem for a distance of six feet, becoming markedly distinct, then fading almost to the natural color of the stem, after which the color became very conspicuous again. Upon cutting into the stem the wood was found to be markedly discolored throughout.

One plant with four shoots each 7 feet tall had one shoot at the base only a few inches in length which had died and turned the color characteristic of the disease. An inspection of the wood showed a few red lines in the crown. The fungus was easily located in this part and was also traced a distance of 20 inches back into the main root. Red lines were also traced into two of the tall shoots for more than a

distance of one foot, although externally they appeared perfectly healthy.

A cane 6 feet 6 inches tall, apparently perfectly healthy, was examined by tearing the stem. The fungus was traceable for more than two feet from the base upward. In many similarly discolored shoots the fungus was traced by following the red lines from 10 to 25 inches and even greater distances. This was done by observing the faint red streaks of the tracheae of the wood.

A large number of tip plants from an infested field were transplanted to a neighboring one having a different type of soil from that in which the plants originally grew. At the time these young plants were ten inches in height many of them began to turn yellow and the leaves began to droop and later many of them turned blue-black throughout the stems and petioles of the leaves. A microscopic examination of the cane showed them to be well filled with the fungus, in fact, in many cases the tracheae were completely plugged by dense masses of the hyphae. (See Fig. 44.)

Characteristics of the Disease as Shown by the Canes.

As a usual thing the canes are associated with shoots which plainly show the disease, although exceptions in which none of the shoots show signs of injury are by no means rare. The following examples serve to give a very good idea of the action of the disease of the canes:

From a hill in which there were five perfectly healthy and vigorous shoots of usual summer growth, but in which the canes did not appear healthy, the fruit began to dry up or become woody. In many cases the berries, by the time they had partially developed in size, became perfectly dry with the exception of the placentae which contained considerable moisture but was very woody. Sections were cut from these canes at various points along their five feet of length. The external layers of the bark did not show the least sign of the blue-black color, but the wood contained many of the reddish lines so characteristic of this disease. In many places the color was unusually faint. Under the microscope, however, an abundance of the mycelium could be seen in the wood, not only where the red lines occurred but in many other places in the stem. In many places even where no discoloration occurred the tracheae were completely filled with a pseudo-parenchymatous tissue. On these canes the leaves on the bases were small, yellow in color but did not show any blackening of the petioles. The crop of fruit from these canes was an entire failure since all of it dried up before picking time. The dried berries, while they retained their original shape appeared much like the dried product, having grown to exceed a height of 8 feet in both shoots and canes and well laden with partially formed berries. Another vigorous hill suddenly stopped growing, the canes dried out without the least signs of discoloration, leaving the fruit dry and hard and still hanging upon the plant. About the time the canes were dead the shoots began to turn blue-black at the base and in a few days were discolored for more than two-thirds their length. A microscopic examination showed the presence of the fungus several inches above the lowest point of discoloration in the shoots while the hyphae could be found in each of the canes as much as 8 feet from the base.

In a field of Parker black raspberries of about one acre, not a single hill could be found in which the canes were not infested. A larger per cent. of them were entirely dead while the remainder did not produce edible fruit, the berries having partially dried up before ma-

turity. Much of the fruit had developed to the size of a field pea while some had died earlier and fallen off. There was no evidence of the disease in the canes except a slight darkening in shade of color. The very strange thing in this field was the presence of only three hills in which the shoots had turned blue-black. An examination under the microscope of a considerable number of canes and shoots plainly showed the presence of the disease since there was an abundance of the hyphae in the canes examined, especially the blue-black ones.

Characteristics of the Disease as Shown by the Roots.

As the fungus works back into the roots from the crown the course may be followed by tracing the reddish or brown discoloration which generally extends throughout the wood zone. The advance into the root system may be confined to one or extend to several of the roots. The progress made varies greatly and is unusually slow. It is not uncommon to find the crop entirely dead with the roots still green or only discolored for a few inches below the point of union with the crown.

DISTRIBUTION AND BEHAVIOR OF DISEASED PLANTS DURING THE YEAR.

More than fifty fields were visited and studied as to the behavior of the different varieties. Sufficient evidence was not gathered to indicate the susceptibility of different varieties since the different forms act so differently. It is evident, however, that the injury to the Cumberland is the most conspicuous and occurs earlier than on other varieties, although the ultimate results are equally disastrous. The method of planting in the field, whether in rows or hills, presents some interesting comparisons. In the rows where plants stand closely together, individual hills when diseased show some marked differences, but as a rule there is less difference in degrees of health and disease than may be observed in the hill system where the individual plants are more widely separated. The only accurate history of the way in which the disease had developed was obtained from Mr. Fred Burglehaus of Sumner. Mr. Burglehaus set 1,006 plants of the Cumberland variety in February, 1903, the plants having been obtained from South Glastenburg, Connecticut. A few, not more than a dozen, of the canes from these tip plants became discolored and died. During the second season 25 per cent. of the remainder died and 25 to 30 per cent. of those remaining died the following season. During the fourth year a special effort was made to prevent further spread of the trouble which was believed to be spreading from the dead and diseased plants. All such plants were removed and burned during early spring time. Just at blooming time the destruction of all

diseased plants was repeated. By the time the fruit was nearly ready for picking a number of plants were dying and for a third time the diseased ones were gathered and burned. About two weeks later the writer visited the field and found eleven hills plainly showing signs of the disease.

After a careful examination of the diseased plants fourteen shoots were selected and the height of discoloration on the stem marked by tying a cord around each. On August 1st, 6th, 11th, and 16th the entire field was examined and the advance of the color of the shoots under observation was marked; and those just showing signs of the disease for the first time, marked and recorded. At the end of the fifteen days a total of fifty-five diseased canes had been observed and the rate of discoloration recorded in a total of 4,013 lineal inches of stem. Twenty-five of these shoots were included in nine hills; while the remainder were scattering, many of them being the first shoot to become discolored in a hill of several canes.

From the data recorded it was found that the rate of advance of discoloration on 42 shoots for a period of five days was 6.6 lineal inches per day. The daily rate of discoloration as observed during a second period of five days for 23 shoots was 46 inches per day, while the rate for the third period of five days for ten canes was but 3.2 inches, with a daily rate of 5.2 inches for all canes for the period of 15 days. Of the 14 canes already discolored a distance of 14 to 50 inches the rate after a period of 15 days for the eight still living was but 3 inches per day. The most rapid discoloration for the period of five days was 14.4 inches per day. The slowest was 1.2 per day. In a few instances no spread occurred in the second period of observation. During these observations the tendency of the rate of discoloration was to decrease. Three cases were recorded, however, where the rate increased while in some there was a marked increase the second period. In a few exceptions there was a marked decrease instead of increase during the second period.

From these and other observations made it is evident that the spread of the fungus in the plants largely occurs during the growing season, the death of plants occurs throughout the entire growing season and that a greater per cent. of them die during the time from the appearance of leaves to the end of harvest time.

THE BEHAVIOR OF THE FUNGUS IN THE FIELD AND IN NON-STERILE CULTURE.

During the entire time field investigations were being conducted, a careful search was made for a perfect stage of the

fungus but none was found. No evidence could be found that the fungus ever produces conidia on the dead cane, even if these be left standing in the field or placed in compost heaps for a period of several months. Cross sections of the stems after the plants had been left in the compost heap for more than a year did not show the formation of chlamydospores which could be

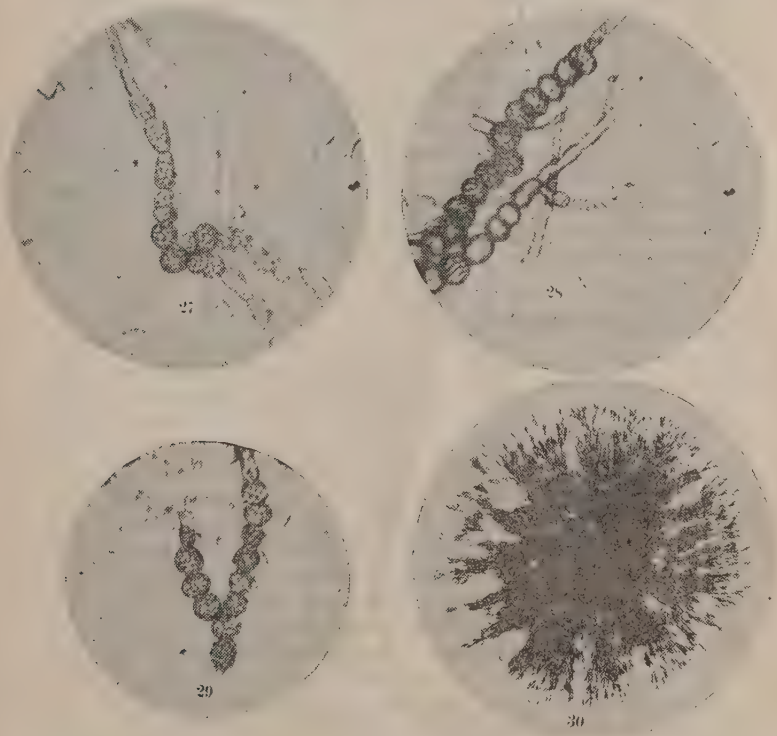


FIG. 27.—Micro-photograph of mycelium, a portion of which has produced a number of chlamydo-spores.

FIG. 28.—Micro-photograph of the mycelium showing two chains of chlamydo-spores.

FIG. 29.—Micro-photograph showing a few well developed chlamydo-spores lying among the branches of the mycelium.

FIG. 30.—A photograph of the mycelium of A. caulophagus which is well filled with chains of chlamydo-spores. The presence of a number of hyaline hyphae throughout except at the center, gives the colony a delicate plumose appearance. The center of the colony was a dense mass of brown mycelium composed largely of chlamydo-spores. This colony shows the usual tendency of concentric growth as shown by the alternate light and dark zones. Photograph by transmitted light, natural size.

obtained abundantly in pure cultures. The behavior of the fungus was quite different, however, when these diseased stems were placed in moist chambers. Conidiospores bearing heads of spores were produced in some abundance especially on cut surfaces. This was particularly true on the injured surfaces of diseased roots, which were known to be well filled with the mycelium. Observations upon short sections of cane placed in water showed the presence of a fringe of mycelium protruding from the cut surfaces within a period of two to five days. The growth amounted to as much as one-half inch in length in many cases and was so dense as to be decidedly conspicuous, giving the cut surface the appearance of being surrounded by a halo. No evidence that the spores form in water could be obtained from these experiments. The crowns of three hills of Cumberland berries were also placed in dirt in the greenhouse and kept moist for a period of three months but no evidences of the conidial or a perfect stage could be found on them during this time.

GROWTH AND ACTION OF FUNGUS IN THE HOST.

The fungus invades all portions of a plant with the possible exception of the blade of the leaf. The first indications of the presence of the fungus may be a distinct change in color of the shoot from the normal to a blue-black, or a yellowing or wilting of the leaves of the shoots (which later may show the more pronounced characters), or a premature drying up of the fruit accompanied by the appearance of red or reddish brown lines in the wood of roots, stems or petioles. The point of infection and the time when infection takes place has not been determined. There is evidence, however, that the fungus lives in the soil and upon entering the plants passes directly through the bark into the wood of the crown, situated below the surface of the ground. Two small diseased portions of roots were found, indicating that the fungus sometimes enters the roots. On two occasions a marked discoloration of the roots of a diseased plant had taken place some distance from the crown. That these infections were distinct was indicated by the presence of normal tissues above and below the areas discolored.

Since the discoloration of the shoot usually occurs first a few inches above the ground, it was at first supposed that the infection takes place in the shoot. But since the red lines show the course of the fungus to be upward from the crown, through the wood, through the discolored area and even to many inches above it, and since the color spreads rapidly through the stem, and cutting off the shoot as soon as the color made its appear-

ance does not stop the spread of the disease, it was concluded that infection must occur below. There does not appear to be any direct relation between the amount and the rate of discoloration and the abundance of the fungus as seen under the microscope. As soon as discoloration begins the coloring matter develops rapidly. At this stage of the disease the shoots die in the shortest time. It would appear that action of the fungus is finally aided by an enzyme which destroys the plant more quickly. The red coloring matter to which the red lines in the wood were due, diffuses readily into water. In diseased roots, the discolored portion often becomes thoroughly saturated with a reddish liquid, a condition which does not occur in the stem.

The fungus can be traced in the roots, petioles of the leaves and in the stems, being usually very abundant in the tracheal tissue. It occurs either as simple, more or less tortuous, slender hyphae or as a much-branched mycelium, the branches of which become strongly curved and interlaced, sometimes developing a pseudo-parenchymateous tissue completely plugging the tracheae. Throughout the root system the fungus is most abundant in the central portion of the wood. In the stem, hyphae, both simple and branched, may be easily seen in all parts of the wood, and even in the outer layers of cells in the pith. (See Figs. 43-44.)

DESCRIPTION OF THE FUNGUS CAUSING "BLUESTEM."

Since the fungus could not be found upon the host in the field nor on dead and decaying plants at a later date, and the growth made upon stems and roots placed in culture chambers was so minute that it was impossible to mount perfect specimens it is necessary to describe the fungus growing in potato-agar as seen under the microscope. Since the fungus proved to be an unknown species of the genus Acrostolagmus, the following specific name and description is proposed by the writer:

ACROSTOLAGMUS CAULOPHAGUS Nov. sp.

Parasitic in the Stems of the Black Raspberry.

Tufts 1 to 3 mm. tall, forming long, floccose, irregular or short thin flaccid and arachnoidal patches; conidiophores hyaline, 100 to 830 mmm. long, non-septate; laterals grouped or single, bearing one or two sets of numerous, short, simple or forked or whorled branches with short ultimate tips slightly attenuated, bearing heads of conidia; mycelium profusely septate, hyaline or olive-brown 3-4 mmm. broad; chlamydospores spherical or oblong, single or in simple or branched chains, 7-12 mmm. in diameter; heads of conidia, hyaline, smooth, round 7.5-25 mmm. usually 8-15 mmm., bearing numerous conidia imbedded in a mucilaginous matrix readily soluble in water; conidia hyaline, oblong to oval, sometimes bigutulate, 3-7, 5 x 2-3 mmm.

OBSERVATIONS ON SPORE FORMATION.

During the observations made upon various species of Acrostolagmus, the peculiar appearance of ultimate tips after heads of spores had fallen off, led to a careful study of spore formation among these species. Figure 24 shows the variations in size and shape of conidia taken from a head of spores. Figure 36 (which represents a two-day old colony from a single spore grown in a sparsely seeded petri-dish containing a medium of potato-agar) shows how rapidly spores are formed in this species (A. caulophagus). In studying these masses of spores it was impossible to discover any of the spores attached to the end of the branches around which they are grouped. In older cultures where moisture had been abundant at earlier periods, some very striking examples of variations occurring in the appearance of ultimate tips, were observed, as shown by Figure 40. Of these three, the tip of one was open and the contents contracted. Of the other two illustrations of this figure, one shows protoplasm emerging from the open tip, while the other represents a conidium just before abstriction.

Owing to the larger size and conspicuous color of *A. cinnabrinaus*, a more satisfactory study in spore formation was made on this species. Figure 47 represents a whorl of five branches all of which were bearing heads of spores. Upon examination of a large number of dry specimens, conditions as represented in Figure 48 were often found. Ultimate tips may be destitute of conidia or may bear them in various stages of formation, even with a conidium still located on the tip but apparently not attached to it. Under the microscope the connection between the tip and conidium appears to be a thin layer of a mucilaginous substance usually more or less variable in thickness, giving it a more or less wrinkled or convoluted surface.

The production of spores by the continuous protrusion of the protoplasmatic contents of the hyphae, is not only common among species of *A crostolagmus*, but in other forms of fungi as illustrated by Figure 49, in which two branches of *Nectria ipomeae Halst.* show that the method of conidial formation in this species is due to the exudation of protoplasm from the open tips of branches.

OBSERVATIONS ON YEAST-LIKE CELLS.

As pointed out elsewhere in this bulletin, the fungus may be induced to change from the filamentous form to a yeast-like one, having also a yeast-like odor. Figure 25, which is a microphotograph of the ascus-like cells, shows the general appearance

of the bodies under the microscope. Each cell or body contains two spore-like objects which appear as two adjacent bodies, the wall enclosing them being very indistinct. Germination tests of these bodies showed that they have the power of reproduction.

Since the spore-like bodies within each ascus-like cell did not show signs of growth, their nature could not be determined, although their general appearance was quite suggestive of the ascospores of yeasts. W. B. Alwood* has previously noted a similar variation in cultures of Sphaeropsis malorium.

"The author reports the discovery of a yeast form in laboratory cultures of the fungus, causing the Black Rot of apples (Sphaeropsis malorium). This yeast form has been isolated and reinfections made on the fruit of apple produced the fruiting bodies characteristic of the Black Rot fungus." (E. S. Rec., Vol. 10, 865.)

THE RELATION OF THE DIFFERENT FORMS OF THE FUNGUS AND THEIR VARIATIONS.

The aerial growth which is long floccose or thin flaccid and arachnoidal is almost white, becoming sordid as soon as the hyphae collapse. These conidiophores are usually 100 to about 900 mm. in length and nonseptate, the ultimate tips occurring in groups of 2 to 5 each, bearing a smooth round head. (See Fig. 37.) These heads vary from 7.5 to 25 mmm. usually 8-15 mmm. and still retain their shape. Larger heads sometimes occur, but these are more or less irregular in shape although occasional ones retain their shape very well up to 40 mmm. Such a condition is only present if moisture has been very abundant at some period and for a comparatively short time. (See Figs. 38, 39 and 46.) The mucus, at such times as the mass becomes too large to bear its weight, becomes irregular and the mass of spores may be seen running down the branches and collecting in tangled masses of dense areas of the fungus or collecting in masses on the substratum. Under culture conditions where there is a good supply of food and the moisture is abundant conidia are produced in such large numbers that the masses become visible to the naked eye as a scale-like mass among the hyphae or a tissue-like growth at the base of the conidiophore.

The conidia vary greatly in size. (See Fig. 24.) The largest may be readily seen in the heads which are composed in part of a hyaline mucus. When the fungus is actively growing and producing good sized heads of conidia, which are in a partially saturated atmosphere, the mucus apparently ab-

* On the occurrence of a yeast form in the Life Cycle of the Black Rot of Apples, W. B. Alwood (Proc. Amer. Assoc. Adv. Sci. 47, p. 422, 1898.)

sorbs moisture, thus increasing in volume. Under such conditions there is usually a very interesting movement of the conidia. The outer film of mucus is smooth, and translucent, a condition present in both large and small heads. The movement of the spores is irregularly intermittent. The conidia, which are at all times buried well within the mucus, usually move on an axis. By looking down upon a number of them, masses of conidia may be seen to spin with great rapidity on a vertical axis, this being the most conspicuous movement. This action may be clockwise or anticlockwise, and intermittent or changeable. The direction of the axis may change to horizontal or to some intermediate position. In addition to these motions there are less frequent but still more interesting streaming movements of the spores in different parts of the head, in many respects resembling the streaming of protoplasm in the cells of *Chara*.

While studying the sizes of spores from different sources, it was observed that when heads of spores are small and few in number, on potato-agar, the heads disappeared at once on touching the water. When grown on potato-lactose-litmus the heads are much larger, contain many spores, and do not separate readily when placed in water. The slow solubility of the mucus surrounding the yeast-like cells developed in Elfving-agar or of the conidiophores bearing heads of spores the same as on the above agar suggests that this action is due to the sugar content of the medium.

Heads of spores apparently have a strong affinity for water since frequently they were apparently thrown apart with considerable force when brought into contact with water.

GERMINATION OF THE CONIDIA.*

Mature conidia germinate within a few hours' time when placed in water. Conidia placed in thin films of potato-agar over which a cover glass was placed, also germinated readily. Germination may be preceded by the conidium becoming slightly swollen and more nearly round. Its contents become slightly granular, after which a germ tube usually makes its appearance at one end. Soon a second tube appears at the opposite end, vacuoles appear in the conidium, the germ tube begins to branch, and oftentimes a third germ tube appears, more commonly near one end of the conidium and close to one

*See Figs. 34, 35, and 36. Fig. 45 illustrates the comparative germination of *A. cinnabarinus*.

of the older germ tubes, especially when the conidium is rather long. This condition is quickly followed by numerous branches from the young mycelium. Toward the margin of the cover glass, conidiophores bearing hyaline conidia irregular in shape and size and slightly larger than those borne in the heads, make their appearance. The short conidiaphores and mycelium become distinctly vacuolate. Near the center of the medium the germ tubes remain short after germination, growth apparently becoming very slow or ceasing. The tendency of many of the conidia in these germination cultures is to reproduce by budding, a characteristic behavior of the fungus as observed in other culture media discussed elsewhere. The behavior near the center of the cultures is believed to be due to a lack of oxygen since other controlling factors were apparently the same.

GROWTH AND CHARACTERS OF THE FUNGUS GROWN ON CULTURE MEDIA.

Ten tubes of potato-agar were inoculated, on July 28th, by removing pieces of diseased tissues from the dead or diseased canes and transferring them to the medium. The fungus grew rapidly in all of the tubes, making its appearance in less than two days' time. On the 30th of August, additional inoculations were made, with the result that all showed the growth of the fungus in the specified time. At various times more than 200 such cultures were made. The fungus failed to appear in pure culture only in three of the entire number and each of these was contaminated with a species of Penicillium. These and other observations suggest that this fungus may produce substances deletory to the growth of similar organisms.

Examinations of the above cultures at the end of 15 to 20 days showed that the fungus had grown into the medium a distance of 2-3 cm. from the point of inoculation. The colony remains circular. On reaching the edge of the medium the hyphae spread over the sides of the test-tube for 5-8 cm. Aerial hyphae grow over the areas as the mycelium progresses, which are either open and distinct or form a dense mass which, while floccose and dense, does not cover the area. The growth of the mycelium up to this time exceeds the growth of conidiophores, the former forming a distinct margin of some width around the latter. At this time the first indications of the formation of chlamydospores is shown by a darkening of the mycelium adjacent to the piece of cane used for inoculating. (See Figs. 27, 28, 29, 30 and 41.)

A careful examination of a large number of the above cultures in October showed the nearly coal-black, round to irregular, patches which had formed at the centers of the cultures, to consist of masses of the mycelium lying just beneath the surface of the medium. At the upper end of each tube, where the agar had become drier at an earlier date, narrow bands extended across the cultures. Below the bands and near the surface of the agar, especially at the margin adjacent to the glass, were small patches of dark hyphae. Such areas were by no means constant throughout the lot of cultures. In nearly every culture, however, in the exposed portion of the medium, and especially where it did not come in contact with the glass, the hyphae showed this characteristic color. The remainder of the hyphae seldom showed discoloration. The discoloration takes place in the order of the mycelial growth.

During this time the areial hyphae gradually become sordid and finally partially collapse forming a dense mat covering the underlying medium. These old cultures showed very great variation in the abundance and rate of growth. (See Fig. 22.)

The fungus as grown in the tube cultures from infested portions of the plants did not produce the conidia in as great abundance as colonies grown from spores seeded in plate cultures.

GROWTH IN DILUTION PLATE CULTURES.

On March 29th, dilution plate cultures were made, using potato-agar as a medium. After five days the small colonies were in some cases one centimeter in diameter. The mycelium in the medium was abundant and the hyphae were growing rapidly, radiating as numerous forked branches of equal prominence, thus giving the entire colony a uniform appearance throughout. The hyphae were septate, 2½ mmm. to 4½ mmm. in diameter, with many of the cells inflated at one end. On April 18th, branching was observed to have taken place at the swollen ends of many of the cells. The hyphae at this time varied in diameter from 5 mmm. to 6 mmm. During the first five days conidia were produced in the agar and were apparently borne in succession on the tips of short branches not unlike the vegetative mycelial branches. These conidia apparently became slightly swollen after becoming detached. Some of them became vacuolate. Germination of some of these conidia took place at once. The masses of conidia and hyphae at the center of these colonies were very conspicuous because of their dense and waxy appearance.

In the older cultures the variation in diameter of the branches of the mycelium is very conspicuous. Near the center of the colony it was not uncommon to observe many 6 mmm. in diameter. Toward the outer portions of the colony the successive branches became smaller, cells longer and the septations very distinct. The later branches of mycelium bearing masses of conidia vary greatly in length. At this time the conidia vary from round to oblong and from 6 mmm. to 10 mmm. in length, among the larger specimens, to minute size. There is a very great variation in size of conidia within the same heads. The smaller ones are much more abundant; they are characterized by having thickened walls at one end and their protoplasmic contents are very thin.

GROWTH OF FUNGUS ON STERILIZED BEANS.

Transfers of the fungus from the dilution plate cultures were made to sterilize beans. This was done in order to secure the growth from a single spore to study its behavior on this sub-stratum. Ten days after the transfers had been made the hyphae were forming a dense layer over the entire substratum. The growth was uniform except at one point about 2 mm. in diameter at which point there were 15 to 20 erect fascicles varying in size from one-half to 3 mm. in height by .5 to .8 mm. in diameter, nearly white (with a light rose tinge), standing so close together that the bases were effused but remaining free above. Each fascicle was nearly cone shaped, and contained a distinct reddish brown central core slightly attenuated near the apex. From these fascicles numerous colorless widely divergent, profusely forked branches were developed. (See Fig. 23.) The ultimate tips of these branches were arranged in whorls of two to five, each bearing a minute round, smooth head. The presence of these many heads seated on the branches gave the entire surface of each fascicle a somewhat powdery appearance.

Examination made at later dates showed that while the fascicles were mature in ten days from the time that cultures were made, at no time during the entire life of the culture did they show a tendency to collapse as did the floccose hyphae over the remainder of the sub-stratum. During its entire growth this culture did not darken the sub-stratum nor did the mycelium become brown.

In order to study the behavior of the fungus on an additional number of bean plug cultures transfers were made by carrying the spores from the head by touching the tips of

some of the branches with a loop of wire containing distilled water. All of the transfers made in this manner were successful pure cultures. Not a single fascicle made its appearance in one of these cultures. The aerial hyphae soon partially collapsed and many of the plugs became very dark in color, as did the repend hyphae of the fungus. The hyphae also showed a wide range in variation of color throughout the same different cultures, the color ranging from dull white to salmon pink and through shades of black.

GROWTH OF THE FUNGUS ON BEAN AGAR.

December 8th three petri dishes each containing 10 c. c. of bean agar were inoculated at the centers by transferring germinating spores from dilution plate cultures. On January 3d, the colonies were 7 to 8 cm. in diameter. Growth at and around the point of inoculation was somewhat more abundant and taller than near the margin. The aerial growth over the remainder of the colonies was uniformly floccose to slightly woolly in appearance.

GROWTH OF THE FUNGUS ON BEAN GELATINE.

On March 21st, test tubes containing this medium were inoculated with the fungus. Six days later the gelatine around the fungus had liquefied but had not changed in color, although the fungus throughout the mycelial portion had changed to a light rose purple. Since the culture was a slope-gelatine which had been inoculated at the upper end and placed in an upright position the liquefied sub-stratum ran down the slope. As the gelatine ran down, it furrowed the sub-stratum to a depth of nearly one-quarter of an inch. On April 1st, a yeast-like growth made its appearance over the entire surface of the gelatine. This growth was apparently due to the large number of conidia having been developed upon the fungus and distributed by the liquefying gelatine. No additional changes took place in this culture up to the time the gelatine had become entirely liquefied, which was May 20th.

On April 4th a series of culture were started by inoculating with germinating spores from a recent plate culture. Five days later the mycelium had become light rose pink with no change in color in the liquid. On April 14th there was a light brown color present in the liquid which had almost doubled in amount. On April 30th, fully half the gelatine had liquefied and the color of the medium was intermediate between vinaceous-rufous and cinnamon-rufous.

GROWTH OF FUNGUS ON POTATO GELATINE.

On March 21st, transfers were made to potato gelatine as above. On the 26th of March, the fungus was making a smooth surface growth, but by April 9th, half of the sub-stratum had become liquefied. There was a distinct rose-purple in the liquid adjacent to the mycelium which was very slow to diffuse either laterally or downward. Five days later fully two-thirds of the sub-stratum had liquefied. On May 20th, all of the sub-stratum had been dissolved without any change in color.

GROWTH OF FUNGUS ON BEEF GELATINE.

The fungus used in this case was a small colony from a single spore. Ten days after the culture was made, strong liquefaction had occurred, without any change in color. Two weeks after the inoculation had been made slight liquefaction had taken place just below the surface but did not continue much beyond the extent of the mycelium. The gelatine in this case became perfectly transparent.

GROWTH OF FUNGUS ON POTATO-LACTOSE-LITMUS.

These cultures were made February 13th. Seven days later, the colonies were 2.2 cm. in diameter. The aerial hyphae were 1 to 2 mm. tall, dense, floccose, very variable in length, giving the colony a shaggy appearance. On February 27th, the mycelium at the center of the colonies became dark colored for a distance of 1.5 to 2 cm.; the growth at the center of the colony became coal black, while the outer portion was either slightly tinged with brown or remained hyaline in color.

GROWTH OF THE FUNGUS IN SUGAR SOLUTIONS.

The fungus was grown for a month in 10% and 20% glucose, 5% and 10% lactose, 10% maltose, 10% fructose and 10%, 20% and 30% sucrose. In all these sugar solutions the fungus made a very poor growth, for the most part clinging to the sides of the tube at the surface and above the liquid. The growth in the liquids was very slight.

GROWTH OF THE FUNGUS ON BEAN BROTH.

Grown for a month in bean broth, the fungus made about the same progress as it did in the sugar solutions.

GROWTH OF THE FUNGUS IN ELFVING SOLUTION.

Elfving solutions 1.5% acid and 6% acid were inoculated and the growth of fungus observed for one month. In the

weak acid solutions growth was weak and entirely below the surface of the liquid. The cells of the mycelium became very much inflated and similar to the yeast-like cells but did not become separated from each other as within elfving agar as described below. Strongly acid elfving solution gave no growth.

GROWTH OF THE FUNGUS ON ELFVING AGAR.

A large series of cultures (75 in number), were made on elfving agar in petri dishes, taking the fungus for inoculation from all conditions under which it was grown in cultures. These sources were: the collapsed and sordid white aerial hyphae from the bean where no fascicles were developed; spores and hyphae from the fascicles produced upon the bean; the coal black hyphae growing in potato agar; mycelium growing directly from a small section of the stem of the diseased black raspberry stem; very short conidiophores grown on potato agar; aerial hyphae grown on litmus agar for a period of twelve days; mycelium from the central portion of a waxy wet, shining yeast-like growth on potato agar; aerial hyphae from the same culture; and the fungus as growing in yeast-like colonies on elfving agar in a petri dish. These 75 cultures were made on April 24th. Regardless of the source of the fungus for inoculation purposes, and of the age of the culture, all of the inoculations were effective, producing a rapid growth of the fungus within the medium. This growth consisted of innumerable spores and numerous short hyphae and multitudes of yeast-like cells, over the surface of which occurred a waxy light brown coat of rather deep irregular folds. These colonies emitted a yeast-like odor.

GROWTH OF THE FUNGUS IN BEEF BOUILLON.

On March 25th, five tubes, each containing about 15 cc. of beef bouillon, were inoculated. Growth of the fungus began at once. By April 9th, there was a slight discoloration of the bouillon throughout, little or no difference in color appeared up to April 23d, at which date the discoloration of the liquid became conspicuous, becoming more intense in parts until May 20th, on which date the liquid varied from almost colorless to a little lighter than orange-rufous. Two days later the bouillon was tested with litmus paper and found to give an alkaline reaction. Three of the cultures were placed in the autoclave and boiled for 20 minutes at 14 and one-half pounds

pressure. This boiling did not produce any change in color of the solution.

INFLUENCES OF CHANGE OF MEDIUM.

As shown in other parts of this article, the filamentous form of the fungus usually occurs on the potato agar, although there is a tendency to produce a yeast-like growth under some conditions. It has also been shown that the usual form of the fungus on elfving agar is the yeast form. The large numbers of variations in cultures of the same and different media show the fungus to be a very variable one.

CHLAMYDOSPORES.

The much branched septate hyaline mycelium as a rule becomes olive-brown in color, a few or all of the cells in a portion or portions of the entire length of a branch or branches round up and become thick walled. The contents of each cell become much greater in volume and coarsely granular. The hyphae are 3 mmm. to 4.5 mmm. in diameter. The spherical to oblong chlamydospores 7 mmm. to 12 mmm. in diameter remain attached in chains. (See Figs. 27 and 28.)

These chlamydospores are produced most abundantly on potato-lactose-litmus and potato-agar, while they are occasionally present in elfving agar and elfving solutions. In potato agar, in which medium the chlamydospores develop best, the hyphae and spores are characterized as given above. Figure 30 gives an excellent illustration of the arrangements of the strands of the chlamydospores and the dark colored mycelial branches as they appear in culture plates of some age. The tendency of the growth of this fungus is quite conspicuous. The center portion is black, outside of which there are more or less alternate, broken, but well defined dark and light rings. This character was very pronounced in all of the cultures in which chlamydospores were formed. In lactose litmus and elfving agars one week old, the colonies were about 2.2 cm. in diameter with aerial hyphae 1 mm. to 2 mm. in height. Seven days later the center portions in the cultures in the lactose-litmus agar were colored black. In an elfving culture the central 1.5 cm. of the colony was hyaline, outside of which the hyphae formed a continuous ring of 1 cm. in width consisting of alternating rings of light and dark greenish black bounded by a hyaline growth 7 mm. in width. In another case where elfving agar was placed in small flasks containing 25 cc. of medium, two cultures showed chains of hyaline chlamydospores, which were

studied in 1.5 acid elfving solutions. Under these conditions, a few cells of hyphae would round up into somewhat the same shape as the large yeast-like cells occurring in elfving agar. These cells rarely become separated, however. A rare variation in the structure of conidiophores in which sporangium-like swellings appear, was also present on this medium.

METHOD OF PROVING THE RELATIONSHIP OF THE FILAMENTOUS FUNGUS AND THE YEAST-LIKE GROWTH.

During the culture studies upon potato agar there appeared at different times, large numbers of spores, in the medium and at the centers of the colonies, noticeable only after the colonies were of some size. In other cases the central portion of the colony presented evidences that the culture had become contaminated with a yeast when the transfer had been made. The variable conditions in the growth of the fungus led the writer to conclude that the differences were entirely due to the peculiar

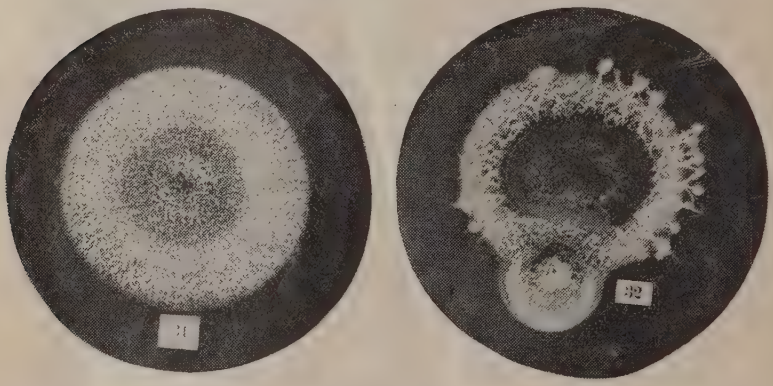
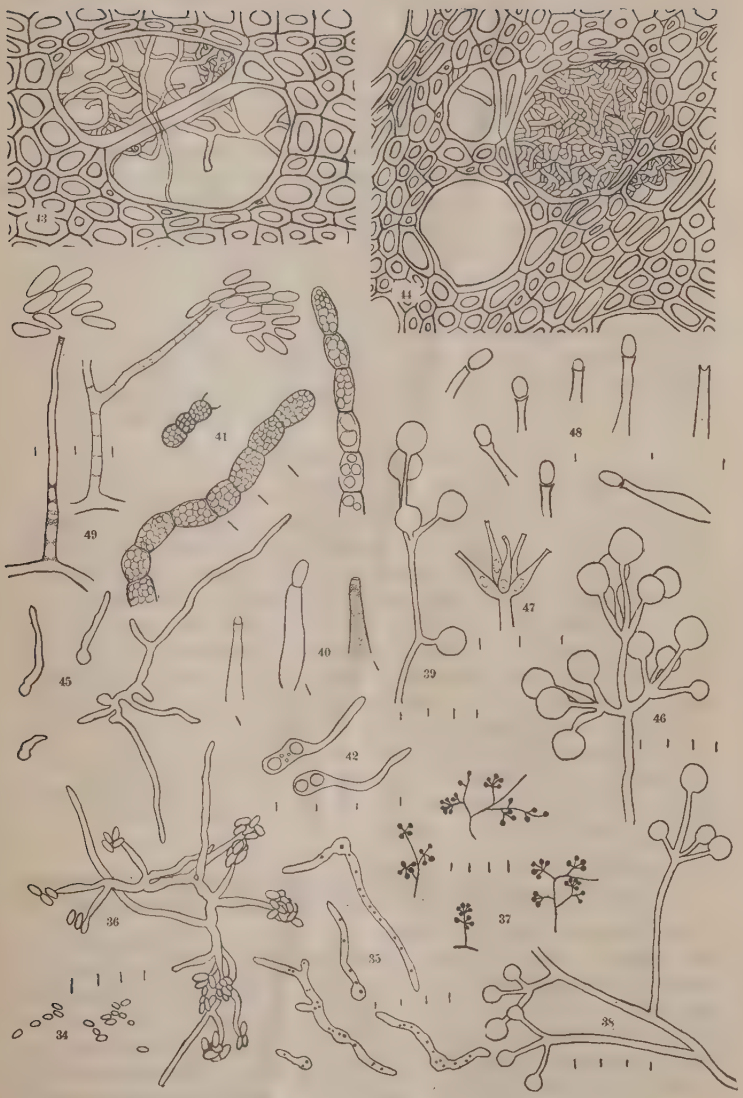


FIG. 31.—Photograph of A. cinnabarinus showing the usual growth made in petri dish cultures with potato agar as the medium.

FIG. 32.—Photograph of A. cinnabarinus showing variations in growth of this fungus on petri dish cultures with potato agar as a medium. The inoculation in this culture was made by transferring a single germinating spore from a dilution plate culture to a petri dish containing the same medium. During the early growth the aerial hyphae were few in numbers and well distributed over the surface. This growth was followed by a spread in the medium with no aerial hyphae produced. This was followed by the formation of a rank growth of fertile hyphae, many of which formed in long, rather compact fascicles standing nearly erect. The presence of moisture carried these fascicles forward and downward until the tips touched the medium. The formation of a dense mycelium at the point of contact is easily observed by noting the plume-like tips of the fascicles as shown in the photograph. The most remarkable variation is shown in the small circular colony over which occurs a dense growth of short woolly conidiophores bearing large numbers of heads of spores. This growth is further characterized by having a mycelium which grows much faster than the fertile hyphae are formed. This variation is shown as a margin which appears much like a halo.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behavior of the fungus rather than contaminations. The fact that 75 petri-plate elfving agar cultures from all possible sources gave the same identical results was good proof that the fungus was induced to change its form when grown under different food environment. To confirm this conclusion, dilution plate cultures were made, using yeast-like cells from the centers of colonies growing in elfving agar. The colonies were eight days old and had all the characteristics of a yeast. In making these dilutions potato agar, potato agar with lactose, litmus added, and the same nutrient with 10 per cent. glucose added, were used. On February 25th, the plates were carefully examined. In every case where growth or germination had taken place the growth was strictly filamentous. In many cases the mycelium had advanced sufficiently to produce masses of spores in the medium. From these experiments it was apparent that the large yeast-like cells germinate readily and produce a more vigorous mycelium in a shorter time than the typical conidia.

YEAST-LIKE FORM.

Early during the study of this fungus large cell-like bodies formed in the agar and water while germination tests were made, and the presence of an occasional similar body in heads of spores led the writer to examine critically a few colonies of this fungus growing on potato agar which had been discarded, on the assumption that they had accidentally become contaminated with the yeast at the time of making the transfers. These cultures at the center portion and occupying as much as 75 to 85 per cent. of the surface, were waxy and shiny and destitute of aerial hyphae and emitted a very distinct yeast-like odor. The medium was well filled with a mixture of large to small conidial-like bodies and disjointed cells or groups of cells which appeared in shape and general appearance like chlamydospores in their earliest stages of formation.

INFLUENCE OF COPPER SULPHATE AND BORDEAUX MIXTURE ON THE PLANTS AND AS PREVENTIVES OF THE DISEASE.

On August 27th, 75 plants were given an application of blue stone solution (2.5 pounds of bluestone to fifty gallons of water), about the roots and crowns of both healthy and diseased plants. Four barrels of the solution were used. There were neither beneficial nor detrimental results from this application. On August 28th fifty plants were treated with four barrels of bordeaux mixture (6-4-50), applied as explained above and with similar results. The resignation of the writer of his connection

with the experiment station has prevented further studies of possible remedial measures.

ACKNOWLEDGMENTS.

The author wishes to express his indebtedness to Professor G. F. Atkinson, Cornell University, for helpful suggestions relative to the laboratory investigations, and to Mr. Fred Burglehaus, his gratitude for the hearty co-operation and assistance rendered in this and other investigations.

DESCRIPTION OF FIGS. 34-49.

I. Acrostolagmus caulophagus.

Fig. 34—Conidia.

Fig. 35—Germinating conidia.

Fig. 36—A two-day old colony from a single spore grown in a sparsely seeded petri dish containing a medium of potato-agar. At this time numerous heads of spores have formed within the medium. Scale, one space equals 10 mmm.

Fig. 37—Camera lucida drawing of four branches of conidiophores showing variations in arrangement and number of branches. Scale, each space equals 50 mmm.

Figs. 38 and 39—Branches of conidiophores showing arrangements of heads of spores. Scale, each space equals 10 mmm.

Fig. 40—Ultimate tips of the conidiophores, showing the method of conidia formation. Of these three illustrations, one shows a tip from which the protoplasm has withdrawn, one a mass of protoplasm just exuding and the third with a well formed conidium. Each space equals 1 mmm.

Fig. 41—Sections of three branches of the mycelium showing the formation of chlamydospores. Each space equals 10 mmm.

Fig. 42—Two yeast-like cells with two spore-like bodies, producing germ-tubes in a water culture. Scale, each space equals 10 mmm.

Fig. 43—Cross-sections of a raspberry stem showing two tracheae in which the mycelium of the fungus is present. Camera lucida drawing.

Fig. 44—Cross-section of a stem of raspberry showing four tracheae, one of which does not contain the fungus, one with a single branch, one with a few branches, while the fourth is completely plugged with a fungus. Camera lucida drawing.

II. Acrostolagmus cinnabarinus.

Fig. 45—Germinating conidia.

Fig. 46—A branch of a conidiophore.

Fig. 47—An ultimate tip of a branch showing the five branches each with an open apex through which the protoplasm exudes, forming the conidia.

Fig. 48—Eight illustrations of the ultimate tips showing the open and characters of the tips and the formation of conidia by the exuding protoplasm.

III. Nectria ipomeae Halst.

Fig. 49—Two branches of this fungus showing the method of conidial formation through the exudation of protoplasm from the open tips of the branches. Each space equals 10 mmm.